

## VARIATION IN LEAF ANATOMY AND CO<sub>2</sub> ASSIMILATION IN *SITANION HYSTRIX* ECOTYPES<sup>1</sup>

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**ABSTRACT.**— Collections of *Sitanion hystrix* known to differ in phenological development, height, dry matter production, and total water use were examined for possible differences in leaf anatomy and in CO<sub>2</sub> assimilation rates. Collections originating in warm, dry habitats produced the narrowest leaves with the fewest veins. Other anatomical characteristics examined were either not different among collections or the differences did not appear to be related to the original habitats. The CO<sub>2</sub> assimilation rates were similar on per-unit weight basis; therefore total assimilation varied as a function of plant size.

Ecotypic variation within plant species has been studied by a number of investigators to better understand how plants adapt to their environment. The ecotype concept, or the genotypic response of a species to environmental factors, had its roots in the 19th-century work of Jordan (Quinn and Ward 1969). Some of the most notable advancements of knowledge were made by Turesson in the 1920s; by Clausen, Keck, and Hiesey in the 1940s; and by McMillan in the 1950s. Ecotypic responses to climate are often through variation in phenology and dry matter production, whereas responses to grazing are often variation in growth forms and phenology. Responses to edaphic variation may be physiological rather than morphological or phenological.

Within grass species, differences occur in numbers of vascular bundles, in stomate density (Dobrenz et al. 1969a, 1969b), and in numbers of mesophyll cells (Wilson and Cooper 1969). Epidermal variations have been noted by Benson and Borrill (1969) and Gray et al. (1969). Photosynthetic and respiration rates have also been shown to vary within species (Klikoff 1968, Wilson and Cooper 1969). For grasses, however, it appears to the author that most within-species variation in internal structure and photosynthesis has been demonstrated among genotypes developed in plant-breeding programs, rather than among naturally occurring races.

The differentiation in response to climatic

variation among *Sitanion hystrix* ecological races occurs in phenological development, height, dry matter production, and respiratory rate, but apparently not in water use efficiency (Klikoff 1968; Clary 1975). The purpose of this study of *Sitanion hystrix* leaf anatomy and CO<sub>2</sub> assimilation rates was to determine whether differences in these characteristics appeared to be ecotypic responses to climate of the collection sites.

### METHODS AND MATERIALS

Clonal plant materials were collected in seven states to obtain samples from a wide variety of habitats and to provide ample opportunity for ecotypic differences to occur among the populations studied. Collections were made in South Dakota, Nebraska, Colorado, New Mexico, Arizona, Utah, and Nevada (Fig. 1). Collection sites varied as much as 13°33' in longitude, 12°14' in latitude, 1600 m in elevation, and 429 mm in annual precipitation (Table 1). These sites represent Merriam's ecological life zones of Upper Sonoran to Hudsonian (Lowe 1964).

The plants were divided into ramets and studied in a transplant garden and a growth chamber. General procedures followed, as well as the study conditions, can be found in Clary (1975).

**LEAF ANATOMY.**— Leaf materials were gathered in the transplant garden during the first week of July for two years. Three leaves,

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one from the center of each of three randomly selected plants, were obtained from individual collections. These were killed and fixed in Craf III solution. The leaf samples were sectioned and stained with a safranin-fast green schedule.

The leaf cross sections were examined microscopically. An ocular micrometer was used to measure three samples per leaf for:

1. Thickness of outer abaxial epidermal wall plus cuticle;
2. Total thickness of abaxial epidermal cells (Fig. 2);
3. Cross-sectional area (height times width) of substomatal cavity;
4. Cross-sectional area (height times width) of bulliform cells; and

#### 5. Width of leaf cross section.

The numbers of leaf veins were recorded in the following categories: (A) veins with a flattened top and a complete column of sclerenchyma fibers from upper to lower epidermis; (B) veins with a rounded top and an incomplete column of supporting tissue; and (C) veins with less than three-quarters the height of A and B category veins with an incomplete column of supporting tissue (Fig. 2). Thirty-five millimeter transparency photographs were taken of the leaf cross sections. These were projected onto a dot grid and the proportion of structural tissue (vascular bundles and sclerenchyma fibers) was determined.

Silicone rubber impressions (Zelitch 1961)

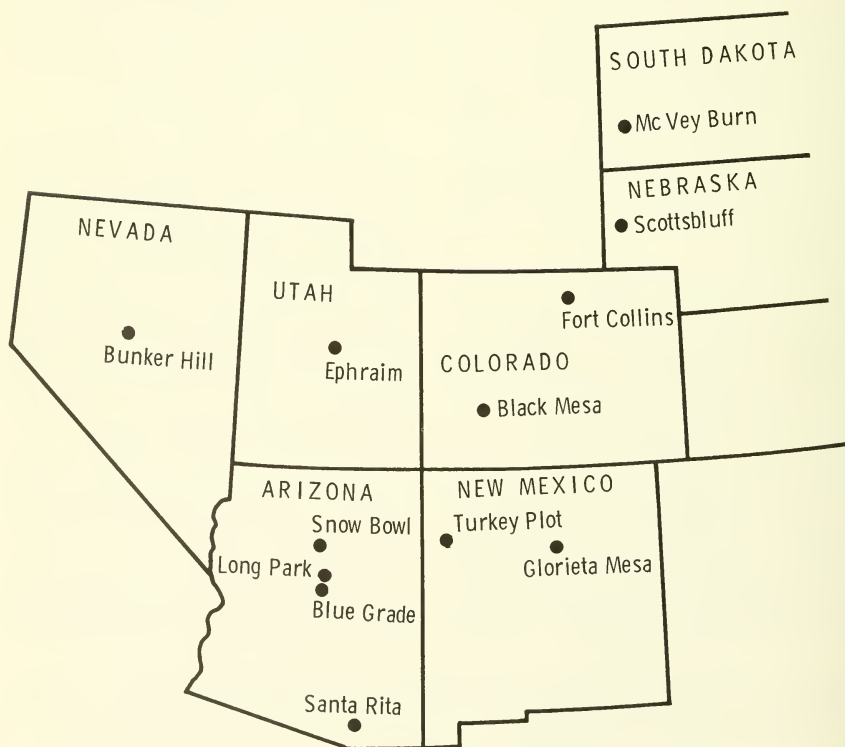


Fig. 1. Geographic distribution of the *Sitanion hystrix* collection sites.

were made of the abaxial leaf surface of three plants in each collection in the transplant garden. The adaxial surface was not used because deep venation obscured the stomata. A transparent positive was obtained by painting the rubber impressions with a thin film of cellulose acetate. Stomate density was determined by counting the number per microscope field. An ocular micrometer was utilized to determine the average length and width of the stomatal apparatus (stoma, guard cells, and subsidiary cells).

**CO<sub>2</sub> ASSIMILATION.**— Fifty-five potted ramets were used in a study of relative rates of CO<sub>2</sub> assimilation. A movable plexiglass assimilation chamber was constructed that could accommodate 10 plants at a time. The chamber was set inside a plant growth room so uniform conditions could be provided. Temperatures inside the assimilation chamber were never lower than 21 C nor higher than 27 C. Each replication was exposed for two hours to 50  $\mu$ C of C<sup>14</sup>O<sub>2</sub>.

Four 2.54-cm leaf sections were immediately taken from each ramet. Alcohol extractions were made from two of the sections and beta particle emissions were counted with a

GM tube and scaler. The remaining two sections were oven dried and weighed. Beta counts per unit weight of leaf were then calculated. The counts were considered an expression of CO<sub>2</sub> assimilation activity.

A second study of relative rates of CO<sub>2</sub> assimilation used *Sitanion hystrix* seedlings because previous observations indicated that plants grown from seed may have more uniform vigor than transplants. Seed was used from four sources: Blue Grade (Arizona), Snow Bowl (Arizona), Long Park (Arizona), and McVey Burn (South Dakota) (Fig. 1). Seventy-two 1.9-liter cartons, 18 per source, were filled with a commercial potting mix and planted with eight seeds. Regular watering maintained a moist substrate. After two weeks, the plants were thinned to four per carton. Assimilation rates were measured at eight weeks.

The procedure for extraction of C<sup>14</sup> from the seedlings was similar to that used for the ramets, except that the entire aerial portions of two plants from each carton were extracted and the aerial portions of the other two plants were oven dried and weighed.

TABLE 1.— Description of *Sitanion hystrix* collection sites.

| Collection site                    | Location          | Elevation (m) | Precipitation (mm) | Climatic index | Merriam life zone |
|------------------------------------|-------------------|---------------|--------------------|----------------|-------------------|
| Snow Bowl (north central Ariz.)    | 111°42'W, 35°20'N | 2,980         | 739                | 0.93           | Hudsonian         |
| Black Mesa (southwest Colo.)       | 107°29'W, 38°30'N | 2,840         | 732                | 1.00           | Hudsonian         |
| Bunker Hill (central Nev.)         | 117°07'W, 39°18'N | 2,620         | —                  | —              | Upper Sonoran     |
| Turkey Plot (northwest N.M.)       | 108°33'W, 35°25'N | 2,380         | 452                | .49            | Transition        |
| Long Park (central Ariz.)          | 111°29'W, 34°53'N | 2,260         | 574                | .59            | Transition        |
| Glorieta Mesa (north central N.M.) | 105°41'W, 35°22'N | 2,200         | 406                | .43            | Upper Sonoran     |
| Ephraim (central Utah)             | 111°36'W, 39°18'N | 1,680         | 310                | .31            | Upper Sonoran     |
| McVey Burn (western S.D.)          | 103°34'W, 44°00'N | 1,520         | 516                | .62            | Transition        |
| Fort Collins (northern Colo.)      | 104°54'W, 40°36'N | 1,520         | 353                | .37            | Upper Sonoran     |
| Blue Grade (central Ariz.)         | 111°43'W, 34°43'N | 1,440         | 396                | .30            | Upper Sonoran     |
| Scottsbluff (western Neb.)         | 103°40'W, 42°00'N | 1,380         | 356                | .38            | Upper Sonoran     |
| Santa Rita (southern Ariz.)        | 110°51'W, 31°46'N | 1,380         | 498                | .30            | Upper Sonoran     |

Joint variable developed from relative growing season length and annual precipitation, and expressed as a proportion of the maximum value

STATISTICAL ANALYSES.— Analyses of variance were computed for leaf anatomy and assimilation data. A modified Tukey comparison (Snedecor 1956) was used where appropriate to isolate significant differences among means.

A climatic index was developed for the collection sites by multiplying an expression of growing season length times annual precipitation, and expressing the result as a proportion of the maximum value. This positioned each collection site on a scale of conditions from warm-dry to cool-wet. The numerical expression of growing season length was calculated by using the transplant garden as base 100. A value of one was added for each day later or subtracted for each day earlier than the transplant garden spring growth would normally be initiated at the collection sites (Hopkins 1918). This procedure produced larger values for short growing seasons and smaller values for long growing seasons. Plant characteristics were studied in relation to the climatic index (Table 1).

## RESULTS

Leaf widths varied significantly among collections. The narrowest leaves occurred in

those collections from warmer and drier sites. Collections of *Sitanion hystrix* from such sites were previously reported to have lesser plant heights (Clary 1975), although the rankings for leaf width and maximum plant height are not highly correlated ( $r=0.59$ ). The relationship of leaf width to characteristics of the original collection sites has not been consistent among other grass species (Quinn 1969, Quinn and Ward 1969).

The collections with the widest leaves generally had the most veins per leaf. The total number of veins and vein categories A and C differed significantly among collections. The total veins per leaf appeared to be quite responsive to conditions of the original collection sites (Fig. 3). Plants from warm, dry habitats had the fewest veins per leaf. The numbers of veins were closely related to the climatic index of the collection sites and followed a consistent pattern in relation to Merriam's life zones.

Collections with the highest number of category A veins (McVey Burn, South Dakota; Long Park, Arizona; Turkey Plot, New Mexico; and Glorieta Mesa, New Mexico) were last to develop phenologically. These veins contain a greater concentration of structural tissue than do B and C category veins; thus, these results may support those of

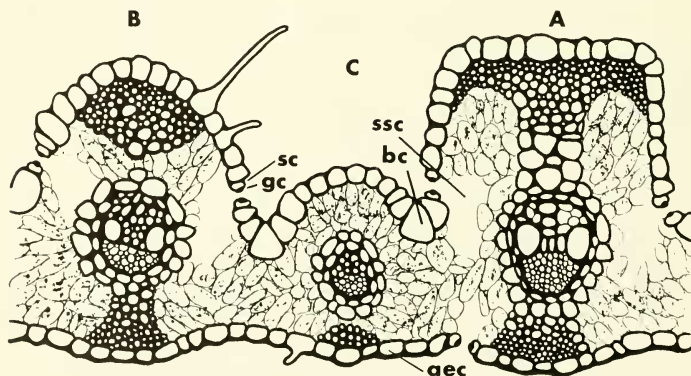


Fig. 2. Typical leaf cross section of *Sitanion hystrix*: A = Category A vein; B = Category B vein; C = Category C vein; aec = abaxial epidermal cell; bc = bulliform cell; gc = guard cell; sc = subsidiary cell; and ssc = sub-stomatal cavity.



and Ward 1969). This may be the result of local genetic drift, particularly for characteristics that have little adaptive importance and are not subject to strong selection pressure.

Carbon dioxide assimilation rates were measured to obtain an index of photosynthetic activity. Among collections, no differences were found in assimilation rates per unit weight of leaf in ramets or seedlings. Assimilation of  $C^{14}$  was very closely related to total weight of seedlings ( $r=0.99$ ).

Assimilation rates were tested under only one set of conditions. Respiration rates have been shown to differ among collections at some temperatures, but not at others (Klikoff 1968). The possibility thus exists that assimilation rates would have varied among collections under different temperature regimens.

#### DISCUSSION

Considerable disagreement exists on the value of variations in leaf structure to  $CO_2$  assimilation rates and efficiency of water use (Ashton 1948, Maximov 1931, Shields 1950, and Milthorpe 1961). The "xeromorphic" leaf form has often been considered important in this regard. This form generally has a low surface to volume ratio, decreased cell size, thicker cell walls, more compact network of veins, higher stomatal frequency, and thickening of the cuticle. The characteristics measured in this study that most typify the xeromorphic leaf form varied little among collections.

In apparent harmony with this lack of xeromorphic trends in arid habitat collections was the similarity among collections of assimilation rates or of water requirements (Clary 1975). The total  $CO_2$  assimilated and  $H_2O$  transpired in a given period did not appear to be a function of specific anatomical or physiological adaptations, but largely a function of plant size, which varied among collections.

A major form of adaptation by *Sitanion hystrix* populations has been to match their timing of growth to the most favorable period of the year, thus reducing the likelihood of severe moisture stress (Clary 1975). Therefore, although the collections were from sites with widely differing climatic conditions,

there may have been little selection pressure on most populations to differentiate a more xeromorphic leaf anatomy.

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